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(54) Title: PLANT ADENYLOSUCCINATE SYNTHETASE AND DNA CODING THEREFOR

(57) Abstract

The present invention provides novel plant DNA sequences coding for native adenylosuccinate synthetase (ADSS). Methods for using the complete or partial ADSS coding sequence as a probe for diagnostic, mapping and other purposes are taught. Generation of transformed host cells capable of expressing ADSS is also taught. Methods of using the transformed host cells are taught, including methods for recombinant production of ADSS enzymes. A method for using the plant ADSS enzyme to screen for inhibitors of ADSS activity is also provided.

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PLANT ADENYLOSUCCINATE SYNTHETASE AND DNA CODING THEREFOR

The invention relates generally to an enzymatic activity involved in adenosine 5'-monophosphate biosynthesis in plants. In particular, the invention relates to the plant enzyme which catalyzes the synthesis of adenylosuccinate and the gene encoding this enzyme. In one aspect, the invention relates to the recombinant production of this enzyme in a heterologous host. In another aspect, the invention is applied to the identification of new herbicides. In yet another aspect, the invention relates to the development of genetic markers in plants.

Adenosine 5'-monophosphate (AMP, also known as adenylic acid) is a precursor of adenosine 5'-triphosphate (ATP), the key energy carrying molecule for all living systems. The first committed enzymatic step in the biosynthesis of AMP is the synthesis of adenylosuccinate from inosine 5'-monophosphate (IMP; inosinic acid) and aspartate. The enzyme which catalyzes this step is known as adenylosuccinate synthetase (IMP:L-aspartate ligase(GDP-forming), EC 6.3.4.4, referred to herein as "ADSS").

In *E. coli*, ADSS is a dimer of identical 48 kD subunits. Its three-dimensional structure has been determined to 2.8 Å resolution (Poland *et al., J. Biol. Chem. 268*:25334-25342 (1993). In mammalian cells, the ADSS enzyme is present as two isoforms. An acidic form, present in non-muscle tissues, is thought to be involved in *de novo* production of AMP. A basic form, present in muscle tissue, thought to act as part of the purine nucelotide cycle, which involves interconversion of IMP and AMP with the net result of deaminating aspartate to fumarate (Lehninger, *Biochemistry*. Worth Publishers, NY (1975), p. 743; Lowenstein, *Int. J. Sports Med.11*: S36-S46 (1990).

Genes encoding the ADSS enzyme have been isolated from a variety of species including *E. coli* (Wolfe and Smith, *J. Biol. Chem. 263:* 19147-19153 (1988)), *D. discoideum* (Weismuller *et al., J. Biol. Chem. 266:* 2480-2485 (1991)), mouse (Guicherit *et al., J. Biol. Chem. 266:* 22582-22587 (1991); Guicherit *et al., J. Biol. Chem. 269:* 4488-4496 (1994), *Bacillus subtilus* (Maentsaelae and Zalkin, *J. Bacteriol. 174:* 1881-1890 (1992), human (Powell *et al., FEBS Lett. 303:* 4-10 (1992), *S. cerevisia* (Genbank accession no. L22185), and *Caenorhabditis elegans* (EST; Genbank accession no. M75738). However, genes encoding the ADSS enzyme have her tofore not been isolated from any plant species.

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Presently, too little is known about the plant ADSS enzyme and its relationship to the ADSS enzymes/genes which have been isolated from other organisms to allow isolation of ADSS encoding genes from any plant species using known approaches.

Methods for isolating genes which are based upon knowledge of the structure of the proteins they encode cannot be applied to plant ADSS genes because too little is presently known about plant ADSS enzymes. Metabolic enzymes such as ADSS are typically very difficult to purify from plants because of their extremely low abundance. In addition, the presence of various phenolic and carbohydrate compounds in plants can interfere with the isolation of pure enzyme with native activity.

In the absence of direct structural information, a number of standard techniques are available for the isolation of proteins and their corresponding genes. Such standard techniques include nucleic acid hybridization and amplification by polymerase chain reaction using oligonucleotide primers corresponding to conserved amino acid sequence motifs. Unfortunately, these techniques would not be expected to be useful for isolation of plant ADSS genes because they rely upon the presence of significant structural similarity (i.e. amino acid and DNA sequence) with known proteins and genes that have the same function. Since there is no significant structural similarity even among the known ADSS genes and proteins from non-plant organisms (*see, e.g.* Powell *et al., FEBS Lett. 303:* 4-10 (1992)) it is unlikely that these proteins would share any significant structural similarity with plant ADSS proteins.

Another approach that has been used to isolate biosynthetic genes in other metabolic pathways from higher eukaryotes is the complementation of microbial mutants deficient in the activity of interest. For this approach, a library of cDNAs from the higher eukaryote is cloned in a vector that can direct expression of the cDNA in th microbial host. The vector is then transformed or otherwise introduced into the mutant microbe, and colonies are selected that are phenotypically no longer mutant.

This strategy has worked for isolating genes from higher eukaryotes that are involved in several metabolic pathways, including histidine biosynthesis (*e.g. see also* International patent application WO 94/26909, incorporated by reference herein in its entirety), lysine biosynthesis (e.g. Frisch *et al., Mol. Gen. Genet. 228*: 287 (1991)), purine biosynthesis (e.g. Aimi *et al., J. Biol. Chem. 265*: 9011 (1990)), and tryptophan biosynthesis (e.g. Niyogi *et al., Plant Cell 5:* 1011 (1993)). This strategy has also been us d to isolate plant gen s including those coding for maiz glutamine synthase (Snustad *et al, Genetics 120:*1111-1114 (1988)), soybean -pyrroline -5-carboxylate reductase (Delaun y *et al., Mol. Genet. 221*:299-305 (1990), maize dihydrodipicolinate synthase (Frisch *et al., Mol. Gen. Genet. 228*:287-293(1991)), rape chloroplast

3-isopropylmalate dehydrogenase (Ellerström *et al., Plant Mol. Biol. 18*:557-566 (1992); Elledge *et al, Proc. Natl. Acad. Sci, USA 88*:1731-1735 (1991)), and dihydroorotate dehydrogenase (Minet *et al., Plant J. 2*:417-422 (1992)).

Microbial mutants thought to be defective in ADSS activity are available (*e.g. E. coli purA* mutant designated CGCS 5408 and *E. coli* strains CGCS 4431 and 7039 from *E. coli* Genetic Stock Center, Yale Univ.; yeast *ade12* mutants reported in Dorfman, *Genetics 61*:377-389 (1969)). However, despite the availability of these mutants, application of the complementation technique to isolate cDNAs encoding ADSS enzymatic activity has proven to be unsuccessful for avian (Powell *et al., FEBS Lett. 303:* 4-10 (1992)) and *B. subtilis* ADSS (Maentsaelae and Zalkin, *J. Bacteriol. 174:* 1881-1890 (1992).

There are several reasons which may explain the failure of this complementation strategy when applied to ADSS, particularly eukaryotic ADSS genes. First, the eukaryotic ADSS cDNA sequence may not be expressed at adequate levels in the mutant microbe, for instance because of codon usage inconsistent with the usage preferences of the microbial host. Second, the primary translation product from the cloned eukaryotic coding sequence may not produce a functional polypeptide, for instance if activity requires a post-translational modification, such as glycosylation, that is not carried out by the microbe. Third, the heterologous protein expressed in *E. coli* may also be lethal to the cells in which it is expressed, thus rendering its isolation impossible. Fourth, the eukaryotic protein may fail to assume its active conformation in the microbial host, for instance if the protein is normally targeted to a specific organellar membrane system that the microbial host specifically lacks. This last possibility is especially likely for the plant ADSS enzyme, which has been associated in the plant cell with organelles not present in microbial hosts used in the complementation assay (Schubert, *Annu. Rev. Plant Physiol.37*:539-574 (1986), and presumably reaches that organellar system as a result of a post-translational targeting mechanism involving both an N-terminal transit sequence, and intrinsic properties of the mature polypeptide (*see, e.g.* Kohorn and Tobin, *Plant Cell 1:* 159 (1989); Li *et al., Plant Cell 3:* 709 (1991); Li *et al., J. Biol. Chem. 267:* 18999 (1992)). Moreover, two other purine biosynthetic genes isolated from plants, 5'-phosphoribosyl-5-aminoimdazole synthetase (Senecoff and Meagher, *Plant Physiol. 102*:387-399 (1993)) and glycinamide synthetase (Schnorr *et al., Plant J. 6*:113-121 (1994)) also appear encode proteins that are targ ted to the chloroplast.

It is thus one of the main objectives of the present invention to identify and isolate DNA molecules encoding an adenylosuccinate synthetase (ADSS) enzyme from a plant source. This objective could be reached within the scope of this invention.

Accordingly, the present invention provides an isolated DNA molecule encoding the adenylosuccinate synthetase (ADSS) enzyme from a plant source.

The DNA coding sequences for ADSS enzymes in *Arabidopsis thaliana, Zea mays* and wheat are provided in SEQ ID NOS: 1, 3 and 5, respectively. Using the information provided by the present invention, the DNA coding sequence for the adenylosuccinate synthetase (ADSS) enzyme from any plant source may now be obtained using standard methods.

The present invention thus relates to an isolated DNA molecule encoding a protein from a plant, preferably from a dicotyledonous or a monocotyledounus plant, more preferably from an *Arabidopsis* species, a maize or a wheat plant, having adenylosuccinate synthetase(ADSS) activity.

In particular, the invention relates to the isolated DNA molecule comprising the coding sequences for ADSS enzymes in *Arabidopsis thaliana, Zea mays* and wheat, which enzymes comprise the amino acid sequence set forth in SEQ ID NO: 2, 4 and 6, respectively.

The invention further relates to an expression cassette comprising a promoter operably linked to a DNA molecule encoding a protein from a plant, preferably from a dicotyledonous or a monocotyledounus plant, more preferably from an *Arabidopsis* species or a maize and wheat plant, having adenylosuccinate synthetase(ADSS) activity.

A further object of the invention is a recombinant vector comprising the said expression cassette wherein said vector is capable of being stably transformed into a host cell. Also comprised is the host cell stably transformed with the said vector wherein said host cell is preferably a cell selected from the group consisting of a bacterial cell, a yeast cell, and an insect cell and is further capable of expressing the DNA molecule according to the invention.

The present invention also encompasses the recombinant production of the ADSS enzyme. In particular, th inv ntion relates to a method of producing a protein having adenylosuccinate synthetas (ADSS) activity in a host organism comprising (a) inserting a DNA sequence encoding a protein having adenylosuccinate synthetase(ADSS) activity into an expression cassette designed for th chosen host;

(b) inserting the resultant molecule, containing the individual elelments linked in proper reading frame, into a vector capable of being transformed into the host cell;
(c) growing the thus transformed host cell in a suitable culture medium; and
(d) isolating the protein product either from the transformed cell or the culture medium or both and purifying it.

Also comprised by the invention are methods for using recombinantly produced ADSS. In particular, the present invention provides methods of using purified ADSS to screen for novel herbicides which affect the activity of ADSS.

Preferred is a method for assaying a chemical for the ability to inhibit the activity of an ADSS enzyme from a plant comprising

(a) combining said ADSS enzyme in a first reaction mixture under conditions in which said ADSS enzyme is capable of catalyzing the synthesis of adenylosuccinate;

(b) combining said chemical and said ADSS enzyme in a second reaction mixture under the same conditions as in said first reaction mixture; and

(d) comparing the amount of adenylosuccinate produced in said first and said second reaction mixture;

wherein said chemical is capable of inhibiting the activity of said ADSS enzyme if the amount of adenylosuccinate in said second reaction mixture is significantly less than the amount of adenylosuccinate in said first reaction mixture.

The present invention is further directed to probes capable of specifically hybridizing to a plant adenylosuccinate synthetase gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an adenylosuccinate synthetase enzyme from a plant at least 10 nucleotides in length.

A further embodiment of the invention is a method of producing a DNA molecule comprising a DNA portion encoding a protein having adenylosuccinate synthetase(ADSS) activity comprising

(a) preparing a nucleotide probe capable of specifically hybridizing to a plant adenylosuccinate synthetase gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an adenylosuccinate synthetase enzyme from a plant at least 10 nucleotides in length;

(b) probing for other ADSS coding sequences in popluations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a); and

(c) isolating a DNA molecule comprising a DNA portion ncoding a protein having adenylosuccinate synthetase(ADSS) activity.

The invention further embodies methods for detecting the presence and form of the ADSS gene and quantitating levels of ADSS transcripts in an organism. These methods may be used to diagnose disease conditions which are associated with an altered form of the ADSS enzyme or altered levels of expression of the ADSS enzyme.

In one aspect, the present invention is directed to an isolated DNA molecule which encodes a eukaryotic form of adenylosuccinate synthetase (referred to herein as "ADSS"), the enzyme which catalyzes the synthesis of adenylosuccinate from IMP. The DNA coding sequence and corresponding amino acid sequence for an ADSS enzyme from *Arabidopsis thaliana* is provided as SEQ ID NOS:1 and 2, respectively. The DNA coding sequence and corresponding amino acid sequence for a maize ADSS enzyme is provided as SEQ ID NOS:3 and 4, respectively. The DNA coding sequence and corresponding amino acid sequence for a wheat ADSS enzyme is provided as SEQ ID NOS:5 and 6,

The DNA encoding the ADSS enzyme may be isolated from the genome of any plant species desired according to the invention. One method taught for isolating a plant ADSS coding sequence is represented by Example 1. In this method cDNA clones encoding an ADSS enzyme are identified from a library of cDNA clones derived from the eukaryote of interest based on their ability to supply ADSS enzymatic activity to a mutant host organism deficient in this activity. Suitable host organisms for use in this method are those which can be used to screen cDNA expression libraries and for which mutants deficient in ADSS activity are either available or can be routinely generated. Such host organisms include, but are not limited to, *E. coli* and yeast.

Alternatively, plant ADSS coding sequences may be isolated according to well known techniques based on their sequence homology to the *Arabidopsis thaliana* (SEQ ID NO:1), *Zea mays* (SEQ ID NO:3) , or wheat (SEQ ID NO:5) ADSS coding sequences taught by the present invention. In these techniques all or part of the known ADSS coding sequence is used as a probe which selectively hybridizes to other ADSS coding sequences present in population of cloned genomic DNA fragments or cDNA fragments (i.e. genomic or cDNA libraries) from a chosen organism. Such techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, *e.g.*. Sambrook *et al.*, "Molecular Cloning" , eds., Cold Spring Harbor Laboratory Press. (1989)) and amplification by PCR using oligonucleotide primers corresponding to s quence domains cons rved among known ADSS amino acid sequences (see, *e.g.* Innis *et al.*, "PCR Protocols, a Guide to Methods and Applications", pub. by Academic Press (1990)). Th s methods are particularly well suited to th isolation of ADSS

coding sequences from organisms closely related to the organism from which the probe sequence is derived. Thus, application of these methods using the *Arabidopsis, Zea mays* or wheat coding sequence as a probe would be expected to be particularly well suited for the isolation of ADSS coding sequences from other plant species.

The isolated plant ADSS sequences taught by the present invention may be manipulated according to standard genetic engineering techniques to suit any desired purpose. For example, the entire ADSS sequence or portions thereof may be used as probes capable of specifically hybridizing to ADSS coding sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among ADSS coding sequences and are at least 10 nucleotides in length, preferably at least 20 nucleotides in length, and most preferably at least 50 nucleotides in length. Such probes may be used to amplify and/or analyze ADSS coding sequences from a chosen organism via the well known process of polymerase chain reaction (PCR). This technique may be useful to isolate additional ADSS coding sequences from a desired organism or as a diagnostic assay to determine the presence of ADSS coding sequences in an organism. This technique may also be used to detect the presence of altered ADSS coding sequences in a plant associated with a particular condition of interest such as herbicide resistance, AMP deficiency, poor health, etc.

ADSS specific hybridization probes may also be used to map the location of the native ADSS gene(s) in the genome of a chosen plant using standard techniques based on the selective hybridization of the probe to genomic ADSS sequences. These techniques include, but are not limited to, identification of DNA polymorphisms identified or contained within the ADSS probe sequence, and use of such polymorphisms to follow segregation of the ADSS gene relative to other markers of known map position in a mapping population derived from self fertilization of a hybrid of two polymorphic parental lines (see e.g. Helentjaris *et al.*, *Plant Mol. Biol. 5:* 109 (1985); Sommer *et al. Biotechniques 12:*82 (1992); D'Ovidio *et al.*, *Plant Mol. Biol. 15:* 169 (1990)). While any plant ADSS sequence is contemplated to be useful as a probe for mapping ADSS genes, preferred probes are those ADSS sequences from plant species more closely related to the chosen plant species, and most preferred probes are those ADSS sequences from the chosen plant species. Mapping of ADSS genes in this manner is contemplated to be particularly useful for breeding purposes. For instanc , by knowing the g netic map position of a mutant ADSS gene that confers h rbicide r sistance, flanking DNA markers can be identified from a refer nce genetic map (see, *e.g.*, Helentjaris, *Trends Genet. 3:* 217 (1987)). During introgression of the

herbicide resistance trait into a new breeding line, these markers can then be used to monitor the extent of ADSS-linked flanking chromosomal DNA still present in the recurrent parent after each round of back-crossing.

ADSS specific hybridization probes may also be used to quantitate levels of ADSS mRNA in a plant using standard techniques such as Northern blot analysis. This technique may be useful as a diagnostic assay to detect altered levels of ADSS expression that may be associated with particular conditions such as deficiencies in adenylosuccinate or AMP levels or enhanced tolerance to herbicides which target ADSS.

For recombinant production of the enzyme in a host organism, the plant ADSS coding sequence may be inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. The choice of specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and enhancer appropriate for the chosen host is within the level of skill of the routineer in the art. The resultant molecule, containing the individual elements linked in proper reading frame, may be inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli* (see, *e.g.* Studier and Moffatt, *J. Mol. Biol. 189:* 113 (1986); Brosius, *DNA 8:* 759 (1989)), yeast (see, *e.g.*, Schneider and Guarente, *Meth. Enzymol. 194*: 373 (1991)) and insect cells (see, *e.g.*, Luckow and Summers, *Bio/Technol. 6:* 47 (1988)). Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), pTrcHis (Invitrogen, La Jolla, CA), and baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV). A preferred baculovirus/insect system is pVI11392/Sf21 cells (Invitrogen, La Jolla, CA).

Recombinantly produced plant ADSS enzyme can be isolated and purified using a variety of standard techniques. The actual techniques which may be used will vary depending upon the host organism used, whether the ADSS enzyme is designed for secretion, and other such factors familiar to the skilled artisan (*see, e.g.* chapter 16 of Ausubel, F. *et al.*, "Current Protocols in Molecular Biology", pub. by John Wiley & Sons, Inc. (1994).

Recombinantly produced plant ADSS enzyme is useful for a vari ty of purposes. For example, it may b used to supply ADSS enzymatic activity *in vitro* to synthesize ad nylosuccinate. *In vitro* synthesis of adenylosuccinate may be accomplished by reacting IMP, GTP, and aspartate in the presence of ADSS enzyme in

an appropriate buffer, containing a divalent cation such as Mg^{2+} (see, *e.g.* Baugher *et al. Biochem. Biophys. Res. Commun. 94*:123-129 (1980); Stayton *et al. Curr. Top. Cell. Regul. 22*:103-141 (1983); Bass *et al., Arch. Biochem. Biophys. 256*:335-342 (1987)). The adenylosuccinate produced is a useful reagent which may be used as a substitute for purified adenylosuccinic acid previously available commercially from other sources.

Recombinantly produced plant ADSS enzyme may also be used in an *in vitro* assay to screen known herbicidal chemicals whose target has not been identified to determine if they inhibit ADSS. Such an *in vitro* assay may also be used as a more general screen to identify chemicals which inhibit ADSS activity and which are therefore herbicide candidates. Alternatively, recombinantly produced ADSS may be used to elucidate the complex structure of this enzyme. Such information regarding the structure of the ADSS enzyme may be used, for example, in the rational design of new inhibitory herbicides.

Typically, the inhibitory effect on ADSS is determined by a reduction or complete inhibition of adenylosuccinate synthesis in the *in vitro* assay (see, *e.g.* Baugher *et al. Biochem. Biophys. Res. Commun. 94*:123-129 (1980); Stayton *et al. Curr. Top. Cell. Regul. 22*:103-141 (1983); Bass *et al., Arch. Biochem. Biophys. 256*:335-342 (1987)). Such a determination may be made simply by comparing the amount of adenylosuccinate synthesized in the *in vitro* assay in the presence and absence of the candidate inhibitor.

A chemical is identified as an ADSS inhibitor if the amount of adenylosuccinate synthetase synthesized in the presence of the chemical is significantly less than the amount synthesized in its absence. The term 'significantly less' is to be understood to refer to a decrease in the amount of ADSS that is less than the margin of error inherent in the measurement technique.

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

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EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory manual, Cold Spring Harbor laboratory, Cold Spring Harbor, NY (1982) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F.M. *et al.*, Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

EXAMPLE 1: Isolation of Arabidopsis cDNAs encoding ADSS genes by functional complementation of an E. coli mutant.

An *Arabidopsis thaliana* (Landsberg) cDNA library in the plasmid vector pFL61 (Minet *et al.*, *Plant J. 2*:417-422 (1992)) was obtained and amplified. The *E. coli purA* mutant PC0543 (CGSC #5408; *E. coli* Genetics Stock Center, Yale University, New Haven, CT) was obtained and maintained on N agar. The plasmid libraries were transformed into CGSC #5408 by electroporation using the Bio-Rad Gene Pulser and the manufacturer's conditions. The cells were plated on minimal E agar (Vogel and Bonner, *J. Biol. Chem. 218*:97-106 (1956) containing 100 mg/ml ampicillin and 0.4% casamino acids at a density of approximately 10,000,000 transformants/10 cm plate. Adenine prototrophs were recovered at a frequency of $1/6x10^7$ from the pFL61 library. Plasmid DNA was isolated from the colony for sequence analysis. Purified plasmid DNA was shown to transform CGSC #5408 to purine prototrophy at high frequency. The purified plasmid complemented two additional *E. coli purA* mutants: ES4 (CGSC #4431; *E. coli* Genetics Stock Center, Yale University, New Haven, CT) and TX595 (CGSC #7039; *E. coli* Genetics Stock Center, Yale University, New Haven, CT), further confirming that it encoded a functional ADSS enzyme.

A restriction digest revealed that the cDNA insert was greater than 3 kB; sequence analysis revealed that the cDNA was chimeric, containing at the 3' end 1512 bp preceded by a polyA region. This 1512 bp region encodes an incomplete ADSS containing the mature protein sequence and a partial probable chloroplast transit peptide. A database search with the GAP program (Deveraux *et al.*, *Nucleic Acids Res. 12*:387-395 (1984) reveals homology with the ADSS from *S. cerevisiae*. The two proteins are 70% similar, 51% identical with regions of high homology. The protein is 65% similar, 44% identical with *E. coli* ADSS.

ADSS-1, in the pBluescript SK vector, was deposited September 22, 1994 with the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A. as pWDC-6 (NRRL #B-21328).

The complete Arabidopsis cDNA sequence encoding ADSS-1 is set forth in SEQ ID NO:1. With the exception of the first four nucleotides, this sequence is contained in pWDC-6 . The ADSS-1 amino acid sequence encoded by this cDNA is set forth in SEQ ID NO: 2.

EXAMPLE 2: Isolation of Maize cDNAs encoding ADSS genes based on sequence homology to Arabidopsis ADSS.

A custom-made Unizap *Zea Mays* (cv. Blizzard) cDNA library was purchased from Clontech. Approximately 160,000 pfu of the phage library was plated at a density of 8,000 plaques per 10 cm Petri dish, and duplicate filter lifts were made onto nitrocellulose membrane (Scheiller and Scheull) after approximately 7 hours growth at 37°C. The filter lifts were probed with a PCR amplified fragment of the Arabidopsis ADSS cDNA labeled with ^{32}P-dCTP by the random priming method (Life Technologies, Bethesda, MD). Hybridization and wash conditions were at 50°C as described in Church and Gilbert, 1984 [*Proc. Natl. Acad. Sci. USA 81:* 1991-1995 (1984)].. After purification to single positively hybridizing plaques, plasmids were *in vivo* excised and cDNA inserts sequenced using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc., Foster City, CA). The sequence thus obtained for the maize ADSS cDNA and the protein it encodes are provided as SEQ ID NOS:3 and 4, respectively. A plasmid containing this maize ADSS cDNA insert was deposited October 24, 1994 as pWDC-9 (NRRL #B-21349).

EXAMPLE 3: Isolation of of Wheat cDNAs encoding ADSS genes based on sequence homology to Maize ADSS

A custom made Unizap Triticum aestivum (cv Kanzler) cDNA library was purchased from Clontech. Approximately 50,000 pfu of the phage library was plated at a density of 5,000 plaques per 10 cm Petri dish, and duplicate filter lifts were made onto nitrocellulose membrane (Scheiller and Scheull) after approximately 7 hours growth at 37° C. The filter lifts were probed with a 1005 base pair EcoRI, Xbal restriction fragment from the 5' nd of th maiz ADSS cDNA labeled with 32P-dCTP

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by the random priming method (Life Technologies, Bethesda, MD). Hybridization and wash conditions were at 50°C as described in Church and Gilbert (1984), *supra*. After purification to single positively hybridizing plaques, plasmids were in vivo excised and cDNA inserts sequenced using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc., Foster City, CA). The sequence thus obtained for the wheat ADSS cDNA and the protein it encodes are provided as SEQ ID NOS: 5 and 6, respectively. This wheat ADSS cDNA is not full-length but it includes the entire coding sequence for the mature ADSS protein which begins at approximately amino acid 35 of SEQ ID NO: 6 based on information obtained by N-terminal sequencing of the mature protein purified from wheat germ. Based on its homology to maize ADSS, the wheat ADSS cDNA lacks coding sequence for nine amino acids of a contemplated chloroplast transit peptide which is not present in the mature protein. A plasmid containing this wheat ADSS cDNA insert was deposited November 3, 1995 as pWDC-10 (NRRL #B-21505).

EXAMPLE 4: Isolation of additional ADSS genes based on sequence homology to known ADSS coding sequences

A phage or plasmid library is plated at a density of approximately 10,000 plaques on a 10 cm Petri dish, and filter lifts of the plaques are made after overnight growth of the plates at 37° C. The plaque lifts are probed with one of the cDNAs set forth in SEQ ID NOS:1, 3, 5, or a portion of such a cDNA exhibiting high sequence conservation among the elucidated plant ADSS sequences. The cDNA probe is labeled with 32P-dCTP by the random priming method by means of a PrimeTime kit (International Biotechnologies, Inc., New Haven, CT). Hybridization conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. After hybridization overnight, the filters are washed with 2X SSC, 1% SDS. Positively hybridizing plaques are detected by autoradiography. After purification to single plaques, cDNA inserts are isolated, and their sequences determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc., Foster City, CA).

The standard experimental protocol described above can be used by one of skill in the art to obtain ADSS genes sequentially homologous to the known ADSS coding sequences from any other eukaryote, particularly other higher plant species.

An alignment of the amino acid s qu nces of the *Arabidopsis*, maize and wheat proteins (SEQ ID NOS: 2,4 and 6, respectively) is set forth in Table 1. An alignment of

the nucleotide sequences encoding these proteins (SEQ ID NOS: 1,3 and 5, respectively) is set forth in Table 2. For each alignment the *Arabidopsis* sequence is used as the reference sequence. Gaps inserted into the sequences to obtain optimal alignment are indicated by dashes. Sequences identical to the *Arabidopsis* sequence in the maize and wheat sequences are denoted by a period and nonidentical sequences are shown.

TABLE 1

Comparison of the Arabidopsis (SEQ ID NO:2and Maize (SEQ ID NO:4), and Wheat (SEQ ID NO:6) S-1 Amino Acid Sequences

Identical residues are denoted by a period. Gaps in the alignment are indicated by a dash.

```
                    10        20        30        40        50
                     *         *         *         *         *
Arabidopsis  MSLSSLTLDSNPRFAVGGPYHRRYPPLHHPRSFVSCS---AKRPAVSASLSVAADSAATE
Maize        ....T.---.H.AA.AA.SGKSLF.AGPAAQ.VHFPK---.RL.VPA.---.S.AT..VH
Wheat        ----------A.AAAGRG.SFS.AAPAP.S.RLPGRQ.PA..AASA.A.E..P..--

             60        70        80        90       100       110
              *         *         *         *         *         *
SLGRIGSLSQVSGVLGCQWGDEGKGKLVDILAQHFDIVARCQGGANAGHTIYNSEGKKFA
AED.VS..T.......S............V..PR..........................
--D.VS.........S............V..PR..........................

120       130       140       150       160       170
  *         *         *         *         *         *
LHLVPSGILNEDTTCVIGNGVVVHLPGLFKEIDGLESNGVSCKGRILVSDRAHLLFDFHQ
.........H.G.L..V...A.I.V..F.G..........R.G.............L..
.........H.G.L..V...A.I.V..F.G.....Q......D.............L..

180       190       200       210       220       230
  *         *         *         *         *         *
EVDGLRESELAKSFIGTTKRGIGPAYSSKVIRNGIRVGDLRHMDTLPQKLDLLLSDAAAR
```

```
A.....A..EN.....C.....T...L..C.....FGD...I.FK...S.
T.....A...N.....C.....T...L..C.....FGD...V.FE.....

240       250       260       270       280       290
  *         *         *         *         *         *
FQGFKYTPEMLREEVEAYKRYADRLEPYITDTVHFINDSISQKKKVLVEGGQATMLDIDF
....Q.SKSL.K....R..KF......F.A....VL.E..K....I.............
.E....SKG..K....R...F.E....F.A....VL.E..R....I.............

300       310       320       330       340       350
  *         *         *         *         *         *
GTYPFVTSSSPSAGGICTGLGIAPSVVGDLIGVVKAYTTRVGSGPFPTENLGTGGDLLRL
.......................RAI..........S..........LF.EE..R..K
.......................R.I.....................L..EE..V..K

360       370       380       390       400       410
  *         *         *         *         *         *
AGQEFGTTTGRPRRCGWLDIVALKFSCQINGFASLNLTKLDVLSDLNEIQLGVAYKRSDG
..M.....................H.......S..........G.S..KV..S.TQT..
..M.....................YC.D....S..........G.P..K...S.NQM..

420       430       440       450       460       470
  *         *         *         *         *         *
TPVKSFPGDLRLLEELHVEYEVLPGWKSDISSVRNYSDLPKAAQQYVERIEELVGVPIHY
QKLQ......DT..QVQ.N.......Q.......R.DE..Q..RL.............V..
EKLQ......DT..QVQ.N.......D.......S..E..Q..RR.........A...V..

480       490
  *         *
IGIGPGRDALIYK
..V..........
..V..........
```

TABLE 2

Comparison of the Arabidopsis (SEQ ID NO:1) Maize (SEQ ID NO:3)and Wheat (SEQ ID NO:5) S-1 Nucleic Acid Sequences in the Coding Region

Identical residues are denoted by a period. Gaps in the alignment are indicated by a dash.

```
                                    40        50        60        70
80        90
                  *         *         *         *         *         *
Arabidopsis       ATGTCTCTCTCTTCCCTCACTCTCGACTCCAATCCAAGATTCGCTGTTGGTGGACCTTAT
Maize             .....G.....CA.A....GC.A.CCGG..GCCG.------...C.CC.--...G.GG.A
Wheat             ----------------GC.G.CGC..C.G..-----------G.GC.GG..A..T....C.

                100       110       120       130       140       150
                  *         *         *         *         *         *
                  CACCGCCGTTATCCTCCTCTTCACCACCCTCGAAGCTTCGTCTCTTGCTCTGCTAAACGT
                  A.T.C.---.T.T.C.GG..GGC..GG.GG..C..-.C...ACA..T.C.CAAGGC...G
                  .C...---------G..G.CC.GG.G..G...TC.G.G..---.C...C.G.GAG.CA.G

                        160       170       180       190       200
                          *         *         *         *         *
                  CCAGCT-----GTCTCCGCTTCACTGAGCG---TCGCCGCTGATTCAGCCGCC-ACTGAG
                  .TCC..-----...C....CG.------..---..T....C.C.A.T..G..T-GT.C.C
                  ..CC.GCCCCC.C.G....G..CGC.CT..CGG.G.AG..G..CC.C......G..A.G.

                210       220       230       240       250       260
                  *         *         *         *         *         *
                  TCTCTTGGACGGATTGGATCACTGAGTCAAGTATCTGGTGTACTCGGTTGCCAATGGGGA
                  G.GGAG.ATA..G..TCG..G....C......C..C..C..G..G..G.CG..G.....C
                  ....-------------G..G.....C..G..C..C..C..G.....G.CG..G.....C

                270       280       290       300       310       320
                  *         *         *         *         *         *
                  GATGAAGGTAAAGGCAAACTCGTTGACATCTTAGCCCAACACTTTGACATCGTTGCTCGT
                  ..C..G..A..G.....G.....C...G.GC.C....CC.G...C.....A..C..G...
                  ..C..G..G..G..G.....C...G.GC.C....CC.G...C........C..G...
```

```
  330        340        350        360        370        380
   *          *          *          *          *          *
TGTCAGGGTGGAGCTAATGCTGGACACACTATATACAATTCAGAGGGAAAGAAATTTGCA
..C.....G.....G..C........T..C..C.....C.....A..C.....G.....T
..C...........A.............C..C.....C..T..A..C...........C

  390        400        410        420        430        440
   *          *          *          *          *          *
CTTCACCTTGTGCCTTCAGGTATCCTGAATGAGGATACTACTTGTGTCATTGGAAACGGA
..G..T.....T..A..T.....T..CC....A.GG..ACTG.....TG....C..T...
.....T.....T..A..T.....T..CC....A.GA..ACTC.....TG....C......

  450        460        470        480        490        500
   *          *          *          *          *          *
GTTGTGGTGCATTTGCCAGGTCTCTTCAAAGAGATTGATGGTTTGGAGTCCAATGGTGTC
.CA..CA.C...G.T.....GT....TGG...A.........C.T...........A...
.CG...A.C...G.T.....GT....TGGC..A.........C.TC.A..A.....A...

  510        520        530        540        550        560
   *          *          *          *          *          *
TCCTGTAAAGGAAGGATTTTGGTCTCTGATCGCGCTCACTTGTTATTCGATTTCCATCAA
CG...CGGT........AC....A..C..C..G..A..TC..C.G..T...C.G..C..G
AGT...G.T.....A..AC....G.....CA.G.....T...C.C..T...C.G.....G

  570        580        590        600        610        620
   *          *          *          *          *          *
GAGGTTGATGGGCTCAGGGAATCTGAGCTTGCCAAGTCGTTCATTGGCACCACCAAGAGG
.CT..G.....A..T......G.A.......AA..T..A..T..A..G..A..T.....A
ACT..A.....A..T......G.C........A..T..C.....A..A..G..T.....A

  630        640        650        660        670        680
   *          *          *          *          *          *
GGAATTGGTCCTGCCTACTCTAGTAAAGTGATAAGGAATGGTATTAGAGTAGGTGATCTC
..C.........TGT.....C..C..G..A.CTC.A.....AC.GC.G..TT.....T.A
..C.....A...TGT..T..C..C..G..C.CTC.A.....GC.GC....TT.......A

  690        700        710        720        730        740
```

```
     *          *          *          *          *          *
AGGCACATGGATACTTTACCTCAAAAGCTTGACCTTTTACTATCAGATGCAGCGGCAAGG
C.A........C.....TGGGG.T.........A.C...T.CAA...C..T..TT.G..A
...........C.....TGGGG.T........TG.....T.CGA......T..T..G...

   750        760        770        780        790        800
     *          *          *          *          *          *
TTTCAAGGGTTCAAGTATACTCCTGAAATGCTTCGGGAAGAAGTTGAAGCATACAAGAGA
........C..TC....C.GCAAAAGCT....CAA......G.....GAG........AG
...G....C........C.GCAAA.GC.....CAA......G.....GAGG........G

   810        820        830        840        850        860
     *          *          *          *          *          *
TACGCTGACAGATTGGAGCCCTACATTACTGACACTGTCCATTTCATCAATGACTCGATT
.TT.....TC.C..........T....G....T..C..G...G.GC.A.....A..T..C
.TT..A..GC.T..........T....G..........T...G.GT.G.....A..C..C

   870        880        890        900        910        920
     *          *          *          *          *          *
TCGCAGAAGAAAAAGGTTTTGGTCGAAGGTGGTCAAGCTACAATGTTGGACATTGACTTT
AA.........G..AA.CC..........C..C.....A..T...C....T.....T...
CGA........G..AA..C....T...........G..A..T...C....T..C..T...

   930        940        950        960        970        980
     *          *          *          *          *          *
GGGACTTATCCTTTTGTTACTTCCTCCAGCCCCTCAGCCGGTGGGATCTGCACAGGTCTT
..C........A.....G.....T..T.....T.....T..C.....A........C..A
..A........A.....G.....T..T.....T..C..T.....A..T.....T..C...

   990       1000       1010       1020       1030       1040
     *          *          *          *          *          *
GGTATTGCACCAAGTGTTGTTGGTGATCTAATTGGAGTGGTAAAAGCATACACTACAAGA
..G.....T.....G.CAA....C..C..G...........C.....T.....AT.T...
..G.....C..T..G...A....C..C..G........T........T.....A.....G

  1050       1060       1070       1080       1090       1100
     *          *          *          *          *          *
```

BNSDOCID: <WO 9619576A1 I >

```
GTTGGTTCAGGTCCATTCCCGACAGAAAATTTGGGCACAGGTGGTGACCTTCTTAGGTTA
..C..C..T..C..T.....A..T...CTA..T..AGAG.AA.....T.GC......AA.
.....C..T..C..T.....A..T...CTGC.T..AGAG.AA.....TG........AAG

  1110       1120       1130       1140       1150       1160
    *          *          *          *          *          *
GCTGGACAGGAGTTTGGCACTACAACTGGTCGTCCTCGTCGGTGTGGCTGGCTTGACATT
......AT...A........A.....A.....C..AA.G..T..C...............
..C...AT...A.....A..G..T..A.....C..AA.A..T.................C

  1170       1180       1190       1200       1210       1220
    *          *          *          *          *          *
GTTGCCCTGAAATTTTCTTGCCAAATCAATGGATTTGCATCACTTAATCTCACTAAGCTT
.....G..T..GCACAGC.............G..CT.............G..C..A..G
.....A.......AC.GC..TG.C........G...T.C..T..A.....A..A..A...

  1230       1240       1250       1260       1270       1280
    *          *          *          *          *          *
GATGTACTTTCGGATCTGAACGAAATCCAGCTGGGTGTGGCTTACAAGAGGAGTGACGGC
.....T..G..C.GGT..TCA.....TA..G.......TT....T.CCCA..C...T..A
.....T..G..C.GGT.ACCA.....TA..........TT....T..TCAA.TG..T..A

  1290       1300       1310       1320       1330       1340
    *          *          *          *          *          *
ACCCCTGTTAAATCATTCCCTGGTGATCTTCGTCTTCTCGAAGAACTGCATGTGGAGTAT
CAGAAGC.GC....C........G......GA.ACC..T..GC..G.A..G..CA.C...
GAGAAAC.AC....C.....A..G......GACACC..G..GC..G.A..G..CA.C...

  1350       1360       1370       1380       1390       1400
    *          *          *          *          *          *
GAAGTCTTACCTGGGTGGAAGTCTGACATATCCTCGGTCAGAAACTACTCTGATCTTCCA
..G..TC.G.........C.AAG......T..T..T..TC...GA...GA...A.....C
..G..GC.T.........G.CAG.........T..T...C...GT...AG...A..C..C

  1410       1420       1430       1440       1450       1460
    *          *          *          *          *          *
AAGGCTGCTCAGCAATATGTTGAGAGGATTGAAGAACTCGTGGGTGTGCCCATTCATTAC
```

```
C.A.....C.GC.TC.....G........A........T..T.....T...G.G..C...
C.A.....C.GC.GT..C..G........A.....G....CC.....T..AG.C..C...

  1470       1480       1490       1500
     *          *          *          *
ATTGGTATTGGGCCCGGTCGTGATGCCCTTATATATAAATGA
......G....A..T..CA.A.....T..C.....C..G.A.
......G.C.....T..GA.G.....T..G.....C..G.A.
```

DEPOSITION

Within the scope of this invention depositions were made with the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A.

A plasmid containing the *Arabidopsis* ADSS-1 cDNA insert provided as SEQ ID NO:1 , was deposited September 22, 1994 as pWDC-6 (NRRL #B-21328).
A plasmid containing the maize ADSS cDNA insert provided as SEQ ID NO:3 was deposited October 24, 1994 as pWDC-9 (NRRL #B-21349).
A plasmid containing the wheat ADSS cDNA insert provided as SEQ ID NO: 6 was deposited November 3, 1995 as pWDC-10 (NRRL #B-21505).

****

Various modifications of the invention described herein will become apparent to those skilled in the art. Such modifications are intended to fall within the scope of the appended claims.

BNSDOCID: <WO 9619576A1 I >

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: CIBA-GEIGY AG

(B) STREET: Klybeckstr. 141

(C) CITY: Basel

(E) COUNTRY: Switzerland

(F) POSTAL CODE (ZIP): 4002

(G) TELEPHONE: +41 61 69 11 11

(H) TELEFAX: + 41 61 696 79 76

(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Plant Adenylosuccinate Synthetase and DNA Coding Therefor

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30B

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1516 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1470
(D) OTHER INFORMATION: /product= "Arabidopsis Adenylosuccinate Synthetase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG TCT CTC TCT TCC CTC ACT CTC GAC TCC AAT CCA AGA TTC GCT GTT      48
Met Ser Leu Ser Ser Leu Thr Leu Asp Ser Asn Pro Arg Phe Ala Val
 1               5                  10                  15

GGT GGA CCT TAT CAC CGC CGT TAT CCT CCT CTT CAC CAC CCT CGA AGC      96
Gly Gly Pro Tyr His Arg Arg Tyr Pro Pro Leu His His Pro Arg Ser
            20                  25                  30

TTC GTC TCT TGC TCT GCT AAA CGT CCA GCT GTC TCC GCT TCA CTG AGC     144
Phe Val Ser Cys Ser Ala Lys Arg Pro Ala Val Ser Ala Ser Leu Ser
        35                  40                  45

GTC GCC GCT GAT TCA GCC GCC ACT GAG TCT CTT GGA CGG ATT GGA TCA     192
Val Ala Ala Asp Ser Ala Ala Thr Glu Ser Leu Gly Arg Ile Gly Ser
    50                  55                  60

CTG AGT CAA GTA TCT GGT GTA CTC GGT TGC CAA TGG GGA GAT GAA GGT     240
Leu Ser Gln Val Ser Gly Val Leu Gly Cys Gln Trp Gly Asp Glu Gly
65                  70                  75                  80

AAA GGC AAA CTC GTT GAC ATC TTA GCC CAA CAC TTT GAC ATC GTT GCT     288
Lys Gly Lys Leu Val Asp Ile Leu Ala Gln His Phe Asp Ile Val Ala
```

BNSDOCID: <WO 9619576A1 I >

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                    85                  90                  95

CGT TGT CAG GGT GGA GCT AAT GCT GGA CAC ACT ATA TAC AAT TCA GAG      336
Arg Cys Gln Gly Gly Ala Asn Ala Gly His Thr Ile Tyr Asn Ser Glu
            100                 105                 110

GGA AAG AAA TTT GCA CTT CAC CTT GTG CCT TCA GGT ATC CTG AAT GAG      384
Gly Lys Lys Phe Ala Leu His Leu Val Pro Ser Gly Ile Leu Asn Glu
        115                 120                 125

GAT ACT ACT TGT GTC ATT GGA AAC GGA GTT GTG GTG CAT TTG CCA GGT      432
Asp Thr Thr Cys Val Ile Gly Asn Gly Val Val Val His Leu Pro Gly
    130                 135                 140

CTC TTC AAA GAG ATT GAT GGT TTG GAG TCC AAT GGT GTC TCC TGT AAA      480
Leu Phe Lys Glu Ile Asp Gly Leu Glu Ser Asn Gly Val Ser Cys Lys
145                 150                 155                 160

GGA AGG ATT TTG GTC TCT GAT CGC GCT CAC TTG TTA TTC GAT TTC CAT      528
Gly Arg Ile Leu Val Ser Asp Arg Ala His Leu Leu Phe Asp Phe His
                165                 170                 175

CAA GAG GTT GAT GGG CTC AGG GAA TCT GAG CTT GCC AAG TCG TTC ATT      576
Gln Glu Val Asp Gly Leu Arg Glu Ser Glu Leu Ala Lys Ser Phe Ile
            180                 185                 190

GGC ACC ACC AAG AGG GGA ATT GGT CCT GCC TAC TCT AGT AAA GTG ATA      624
Gly Thr Thr Lys Arg Gly Ile Gly Pro Ala Tyr Ser Ser Lys Val Ile
        195                 200                 205

AGG AAT GGT ATT AGA GTA GGT GAT CTC AGG CAC ATG GAT ACT TTA CCT      672
Arg Asn Gly Ile Arg Val Gly Asp Leu Arg His Met Asp Thr Leu Pro
    210                 215                 220

CAA AAG CTT GAC CTT TTA CTA TCA GAT GCA GCG GCA AGG TTT CAA GGG      720
Gln Lys Leu Asp Leu Leu Leu Ser Asp Ala Ala Ala Arg Phe Gln Gly
225                 230                 235                 240
```

```
TTC AAG TAT ACT CCT GAA ATG CTT CGG GAA GAA GTT GAA GCA TAC AAG      768
Phe Lys Tyr Thr Pro Glu Met Leu Arg Glu Glu Val Glu Ala Tyr Lys
                245                 250                 255

AGA TAC GCT GAC AGA TTG GAG CCC TAC ATT ACT GAC ACT GTC CAT TTC      816
Arg Tyr Ala Asp Arg Leu Glu Pro Tyr Ile Thr Asp Thr Val His Phe
            260                 265                 270

ATC AAT GAC TCG ATT TCG CAG AAG AAA AAG GTT TTG GTC GAA GGT GGT      864
Ile Asn Asp Ser Ile Ser Gln Lys Lys Lys Val Leu Val Glu Gly Gly
        275                 280                 285

CAA GCT ACA ATG TTG GAC ATT GAC TTT GGG ACT TAT CCT TTT GTT ACT      912
Gln Ala Thr Met Leu Asp Ile Asp Phe Gly Thr Tyr Pro Phe Val Thr
    290                 295                 300

TCC TCC AGC CCC TCA GCC GGT GGG ATC TGC ACA GGT CTT GGT ATT GCA      960
Ser Ser Ser Pro Ser Ala Gly Gly Ile Cys Thr Gly Leu Gly Ile Ala
305                 310                 315                 320

CCA AGT GTT GTT GGT GAT CTA ATT GGA GTG GTA AAA GCA TAC ACT ACA     1008
Pro Ser Val Val Gly Asp Leu Ile Gly Val Val Lys Ala Tyr Thr Thr
                325                 330                 335

AGA GTT GGT TCA GGT CCA TTC CCG ACA GAA AAT TTG GGC ACA GGT GGT     1056
Arg Val Gly Ser Gly Pro Phe Pro Thr Glu Asn Leu Gly Thr Gly Gly
            340                 345                 350

GAC CTT CTT AGG TTA GCT GGA CAG GAG TTT GGC ACT ACA ACT GGT CGT     1104
Asp Leu Leu Arg Leu Ala Gly Gln Glu Phe Gly Thr Thr Thr Gly Arg
        355                 360                 365

CCT CGT CGG TGT GGC TGG CTT GAC ATT GTT GCC CTG AAA TTT TCT TGC     1152
Pro Arg Arg Cys Gly Trp Leu Asp Ile Val Ala Leu Lys Phe Ser Cys
    370                 375                 380
```

BNSDOCID: <WO 9619576A1 I >

```
CAA ATC AAT GGA TTT GCA TCA CTT AAT CTC ACT AAG CTT GAT GTA CTT      1200
Gln Ile Asn Gly Phe Ala Ser Leu Asn Leu Thr Lys Leu Asp Val Leu
385                 390                 395                 400

TCG GAT CTG AAC GAA ATC CAG CTG GGT GTG GCT TAC AAG AGG AGT GAC      1248
Ser Asp Leu Asn Glu Ile Gln Leu Gly Val Ala Tyr Lys Arg Ser Asp
                405                 410                 415

GGC ACC CCT GTT AAA TCA TTC CCT GGT GAT CTT CGT CTT CTC GAA GAA      1296
Gly Thr Pro Val Lys Ser Phe Pro Gly Asp Leu Arg Leu Leu Glu Glu
            420                 425                 430

CTG CAT GTG GAG TAT GAA GTC TTA CCT GGG TGG AAG TCT GAC ATA TCC      1344
Leu His Val Glu Tyr Glu Val Leu Pro Gly Trp Lys Ser Asp Ile Ser
        435                 440                 445

TCG GTC AGA AAC TAC TCT GAT CTT CCA AAG GCT GCT CAG CAA TAT GTT      1392
Ser Val Arg Asn Tyr Ser Asp Leu Pro Lys Ala Ala Gln Gln Tyr Val
    450                 455                 460

GAG AGG ATT GAA GAA CTC GTG GGT GTG CCC ATT CAT TAC ATT GGT ATT      1440
Glu Arg Ile Glu Glu Leu Val Gly Val Pro Ile His Tyr Ile Gly Ile
465                 470                 475                 480

GGG CCC GGT CGT GAT GCC CTT ATA TAT AAA TGATTTTTAG TGTTAGGCTT      1490
Gly Pro Gly Arg Asp Ala Leu Ile Tyr Lys
                485                 490

TTTTGGTTCC TCCACAAACT CAAAAT                                       1516
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 490 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ser Leu Ser Ser Leu Thr Leu Asp Ser Asn Pro Arg Phe Ala Val
 1               5                  10                  15

Gly Gly Pro Tyr His Arg Arg Tyr Pro Pro Leu His His Pro Arg Ser
            20                  25                  30

Phe Val Ser Cys Ser Ala Lys Arg Pro Ala Val Ser Ala Ser Leu Ser
        35                  40                  45

Val Ala Ala Asp Ser Ala Ala Thr Glu Ser Leu Gly Arg Ile Gly Ser
    50                  55                  60

Leu Ser Gln Val Ser Gly Val Leu Gly Cys Gln Trp Gly Asp Glu Gly
65                  70                  75                  80

Lys Gly Lys Leu Val Asp Ile Leu Ala Gln His Phe Asp Ile Val Ala
                85                  90                  95

Arg Cys Gln Gly Gly Ala Asn Ala Gly His Thr Ile Tyr Asn Ser Glu
            100                 105                 110

Gly Lys Lys Phe Ala Leu His Leu Val Pro Ser Gly Ile Leu Asn Glu
        115                 120                 125

Asp Thr Thr Cys Val Ile Gly Asn Gly Val Val Val His Leu Pro Gly
    130                 135                 140

Leu Phe Lys Glu Ile Asp Gly Leu Glu Ser Asn Gly Val Ser Cys Lys
145                 150                 155                 160

Gly Arg Ile Leu Val Ser Asp Arg Ala His Leu Leu Phe Asp Phe His
                165                 170                 175
```

```
Gln Glu Val Asp Gly Leu Arg Glu Ser Glu Leu Ala Lys Ser Phe Ile
            180                 185                 190

Gly Thr Thr Lys Arg Gly Ile Gly Pro Ala Tyr Ser Ser Lys Val Ile
        195                 200                 205

Arg Asn Gly Ile Arg Val Gly Asp Leu Arg His Met Asp Thr Leu Pro
    210                 215                 220

Gln Lys Leu Asp Leu Leu Leu Ser Asp Ala Ala Ala Arg Phe Gln Gly
225                 230                 235                 240

Phe Lys Tyr Thr Pro Glu Met Leu Arg Glu Glu Val Glu Ala Tyr Lys
                245                 250                 255

Arg Tyr Ala Asp Arg Leu Glu Pro Tyr Ile Thr Asp Thr Val His Phe
            260                 265                 270

Ile Asn Asp Ser Ile Ser Gln Lys Lys Lys Val Leu Val Glu Gly Gly
        275                 280                 285

Gln Ala Thr Met Leu Asp Ile Asp Phe Gly Thr Tyr Pro Phe Val Thr
    290                 295                 300

Ser Ser Ser Pro Ser Ala Gly Gly Ile Cys Thr Gly Leu Gly Ile Ala
305                 310                 315                 320

Pro Ser Val Val Gly Asp Leu Ile Gly Val Val Lys Ala Tyr Thr Thr
                325                 330                 335

Arg Val Gly Ser Gly Pro Phe Pro Thr Glu Asn Leu Gly Thr Gly Gly
            340                 345                 350

Asp Leu Leu Arg Leu Ala Gly Gln Glu Phe Gly Thr Thr Thr Gly Arg
        355                 360                 365
```

Pro Arg Arg Cys Gly Trp Leu Asp Ile Val Ala Leu Lys Phe Ser Cys
370 375 380

Gln Ile Asn Gly Phe Ala Ser Leu Asn Leu Thr Lys Leu Asp Val Leu
385 390 395 400

Ser Asp Leu Asn Glu Ile Gln Leu Gly Val Ala Tyr Lys Arg Ser Asp
405 410 415

Gly Thr Pro Val Lys Ser Phe Pro Gly Asp Leu Arg Leu Leu Glu Glu
420 425 430

Leu His Val Glu Tyr Glu Val Leu Pro Gly Trp Lys Ser Asp Ile Ser
435 440 445

Ser Val Arg Asn Tyr Ser Asp Leu Pro Lys Ala Ala Gln Gln Tyr Val
450 455 460

Glu Arg Ile Glu Glu Leu Val Gly Val Pro Ile His Tyr Ile Gly Ile
465 470 475 480

Gly Pro Gly Arg Asp Ala Leu Ile Tyr Lys
485 490

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1835 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 18..1469

(D) OTHER INFORMATION: /product= "Maize Adenylosuccinate Synthetase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AAAACCCTCC CACCATC ATG TCG CTC TCC ACA CTC AGC CAC CCG GCC GCC          50
                   Met Ser Leu Ser Thr Leu Ser His Pro Ala Ala
                                   495                 500

GCC GCC GCC GGG AGC GGA AAA TCC CTT TTC CCG GCT GGC CCG GCG GCG          98
Ala Ala Ala Gly Ser Gly Lys Ser Leu Phe Pro Ala Gly Pro Ala Ala
            505                 510                 515

CAG TCC GTA CAT TTC CCC AAG GCA CGG CTC CCT GTC CCC GCC GCC GTC         146
Gln Ser Val His Phe Pro Lys Ala Arg Leu Pro Val Pro Ala Ala Val
        520                 525                 530

TCC GCC GCT ACT GCG GCT GTT CAC GCG GAG GAT AGG GTT TCG TCG CTG         194
Ser Ala Ala Thr Ala Ala Val His Ala Glu Asp Arg Val Ser Ser Leu
    535                 540                 545

ACT CAA GTC TCC GGC GTG CTG GGG TCG CAG TGG GGC GAC GAG GGA AAG         242
Thr Gln Val Ser Gly Val Leu Gly Ser Gln Trp Gly Asp Glu Gly Lys
550                 555                 560                 565

GGC AAG CTC GTC GAC GTG CTC GCC CCC CGC TTC GAC ATA GTC GCG CGT         290
Gly Lys Leu Val Asp Val Leu Ala Pro Arg Phe Asp Ile Val Ala Arg
                570                 575                 580

TGC CAG GGG GGA GCG AAC GCT GGA CAT ACC ATC TAC AAC TCA GAA GGC         338
Cys Gln Gly Gly Ala Asn Ala Gly His Thr Ile Tyr Asn Ser Glu Gly
            585                 590                 595
```

```
AAG AAG TTT GCT CTG CAT CTT GTT CCA TCT GGT ATT CTC CAT GAA GGG      386
Lys Lys Phe Ala Leu His Leu Val Pro Ser Gly Ile Leu His Glu Gly
        600                 605                 610

ACA CTG TGT GTT GTT GGC AAT GGA GCA GTC ATC CAT GTT CCA GGG TTC      434
Thr Leu Cys Val Val Gly Asn Gly Ala Val Ile His Val Pro Gly Phe
    615                 620                 625

TTT GGA GAA ATT GAT GGT CTT GAG TCC AAT GGA GTC CGC TGC GGT GGA      482
Phe Gly Glu Ile Asp Gly Leu Glu Ser Asn Gly Val Arg Cys Gly Gly
630                 635                 640                 645

AGG ATA CTG GTA TCC GAC CGG GCA CAT CTG CTG TTT GAT CTG CAC CAG      530
Arg Ile Leu Val Ser Asp Arg Ala His Leu Leu Phe Asp Leu His Gln
                650                 655                 660

GCT GTG GAT GGA CTT AGG GAA GCA GAG CTT GAA AAT TCA TTT ATA GGG      578
Ala Val Asp Gly Leu Arg Glu Ala Glu Leu Glu Asn Ser Phe Ile Gly
            665                 670                 675

ACA ACT AAG AGA GGC ATT GGT CCT TGT TAC TCC AGC AAG GTA ACT CGA      626
Thr Thr Lys Arg Gly Ile Gly Pro Cys Tyr Ser Ser Lys Val Thr Arg
        680                 685                 690

AAT GGA CTG CGG GTT TGT GAT TTA CGA CAC ATG GAC ACT TTT GGG GAT      674
Asn Gly Leu Arg Val Cys Asp Leu Arg His Met Asp Thr Phe Gly Asp
    695                 700                 705

AAG CTT GAC ATC TTA TTC AAA GAC GCT GCT TCG AGA TTT CAA GGC TTT      722
Lys Leu Asp Ile Leu Phe Lys Asp Ala Ala Ser Arg Phe Gln Gly Phe
710                 715                 720                 725

CAG TAC AGC AAA AGC TTG CTC AAG GAA GAG GTT GAG AGA TAC AAG AAG      770
Gln Tyr Ser Lys Ser Leu Leu Lys Glu Glu Val Glu Arg Tyr Lys Lys
                730                 735                 740

TTT GCT GAT CGC TTG GAG CCC TTC ATT GCT GAT ACC GTG CAT GTG CTA      818
```

```
Phe Ala Asp Arg Leu Glu Pro Phe Ile Ala Asp Thr Val His Val Leu
            745                 750                 755

AAT GAA TCT ATC AAG CAG AAG AAG AAA ATC CTG GTC GAA GGC GGC CAA      866
Asn Glu Ser Ile Lys Gln Lys Lys Lys Ile Leu Val Glu Gly Gly Gln
        760                 765                 770

GCA ACT ATG CTG GAT ATT GAT TTT GGC ACT TAT CCA TTT GTG ACT TCT      914
Ala Thr Met Leu Asp Ile Asp Phe Gly Thr Tyr Pro Phe Val Thr Ser
    775                 780                 785

TCT AGC CCT TCA GCT GGC GGG ATA TGC ACA GGC CTA GGG ATT GCT CCA      962
Ser Ser Pro Ser Ala Gly Gly Ile Cys Thr Gly Leu Gly Ile Ala Pro
790                 795                 800                 805

AGG GCA ATT GGC GAC CTG ATT GGA GTG GTC AAA GCT TAC ACA TCT AGA     1010
Arg Ala Ile Gly Asp Leu Ile Gly Val Val Lys Ala Tyr Thr Ser Arg
                810                 815                 820

GTC GGC TCT GGC CCT TTC CCA ACT GAA CTA TTT GGA GAG GAA GGT GAT     1058
Val Gly Ser Gly Pro Phe Pro Thr Glu Leu Phe Gly Glu Glu Gly Asp
            825                 830                 835

CGC CTT AGG AAA GCT GGA ATG GAA TTT GGC ACA ACA ACA GGT CGC CCA     1106
Arg Leu Arg Lys Ala Gly Met Glu Phe Gly Thr Thr Thr Gly Arg Pro
        840                 845                 850

AGG CGT TGC GGC TGG CTT GAC ATT GTT GCG CTT AAG CAC AGC TGC CAA     1154
Arg Arg Cys Gly Trp Leu Asp Ile Val Ala Leu Lys His Ser Cys Gln
    855                 860                 865

ATC AAT GGG TTC TCA TCA CTT AAT CTG ACC AAA CTG GAT GTT CTG TCC     1202
Ile Asn Gly Phe Ser Ser Leu Asn Leu Thr Lys Leu Asp Val Leu Ser
870                 875                 880                 885

GGG TTG TCA GAA ATT AAG GTG GGT GTT TCT TAT ACC CAG ACT GAT GGA     1250
Gly Leu Ser Glu Ile Lys Val Gly Val Ser Tyr Thr Gln Thr Asp Gly
```

BNSDOCID: <WO 9619576A1 I >

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                890                 895                 900

CAG AAG CTG CAA TCC TTC CCT GGG GAT CTT GAT ACC CTT GAG CAA GTA      1298
Gln Lys Leu Gln Ser Phe Pro Gly Asp Leu Asp Thr Leu Glu Gln Val
            905                 910                 915

CAG GTC AAC TAT GAG GTT CTG CCT GGG TGG CAA AGT GAC ATT TCT TCT      1346
Gln Val Asn Tyr Glu Val Leu Pro Gly Trp Gln Ser Asp Ile Ser Ser
        920                 925                 930

GTT CGA AGA TAC GAT GAA CTT CCC CAA GCT GCC CGC CTC TAT GTG GAG      1394
Val Arg Arg Tyr Asp Glu Leu Pro Gln Ala Ala Arg Leu Tyr Val Glu
    935                 940                 945

AGG ATA GAA GAA CTT GTT GGT GTT CCC GTG CAC TAC ATT GGT GTT GGA      1442
Arg Ile Glu Glu Leu Val Gly Val Pro Val His Tyr Ile Gly Val Gly
950                 955                 960                 965

CCT GGC AGA GAT GCT CTC ATA TAC AAG TAAAAGCAAC TTTATTTGGT           1489
Pro Gly Arg Asp Ala Leu Ile Tyr Lys
                970

CCTTGGTTGG GCGGAAACCT GGCCGGGACT CGGGAGCATT TGCATTTTCT TGGCGTGGTA   1549

GCTTTTGATA CGGTGAAGTC ACTGACTCGT GGAGTGATGT TGCTCAATAA TCAGAACCTT   1609

GTTCTAATAC AGCCGCTGAG ACATCAGCTA AGGCGAATAA GGGAAGGATG AGTCATTTGC   1669

ACCATGTTTG ACCACCAATT GTTAGGTGGT CCATATATTT TGTACTAATT GTGAGACTTT   1729

GTGCTATGGA TCTCAACTGT ATACCTTGCT GGTGCATGGC TTTGGGTTTA CATGGTTGAA   1789

AATGAGATTG GTGTACTAAT TGTCTAAAAA AAAAAAAAAA AAAAA                   1835
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 484 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Leu Ser Thr Leu Ser His Pro Ala Ala Ala Ala Ala Gly Ser
 1               5                  10                  15

Gly Lys Ser Leu Phe Pro Ala Gly Pro Ala Ala Gln Ser Val His Phe
            20                  25                  30

Pro Lys Ala Arg Leu Pro Val Pro Ala Ala Val Ser Ala Ala Thr Ala
        35                  40                  45

Ala Val His Ala Glu Asp Arg Val Ser Ser Leu Thr Gln Val Ser Gly
    50                  55                  60

Val Leu Gly Ser Gln Trp Gly Asp Glu Gly Lys Gly Lys Leu Val Asp
65                  70                  75                  80

Val Leu Ala Pro Arg Phe Asp Ile Val Ala Arg Cys Gln Gly Gly Ala
                85                  90                  95

Asn Ala Gly His Thr Ile Tyr Asn Ser Glu Gly Lys Lys Phe Ala Leu
            100                 105                 110

His Leu Val Pro Ser Gly Ile Leu His Glu Gly Thr Leu Cys Val Val
        115                 120                 125

Gly Asn Gly Ala Val Ile His Val Pro Gly Phe Phe Gly Glu Ile Asp
    130                 135                 140

Gly Leu Glu Ser Asn Gly Val Arg Cys Gly Gly Arg Ile Leu Val Ser
```

145 150 155 160

Asp Arg Ala His Leu Leu Phe Asp Leu His Gln Ala Val Asp Gly Leu
165 170 175

Arg Glu Ala Glu Leu Glu Asn Ser Phe Ile Gly Thr Thr Lys Arg Gly
180 185 190

Ile Gly Pro Cys Tyr Ser Ser Lys Val Thr Arg Asn Gly Leu Arg Val
195 200 205

Cys Asp Leu Arg His Met Asp Thr Phe Gly Asp Lys Leu Asp Ile Leu
210 215 220

Phe Lys Asp Ala Ala Ser Arg Phe Gln Gly Phe Gln Tyr Ser Lys Ser
225 230 235 240

Leu Leu Lys Glu Glu Val Glu Arg Tyr Lys Lys Phe Ala Asp Arg Leu
245 250 255

Glu Pro Phe Ile Ala Asp Thr Val His Val Leu Asn Glu Ser Ile Lys
260 265 270

Gln Lys Lys Lys Ile Leu Val Glu Gly Gly Gln Ala Thr Met Leu Asp
275 280 285

Ile Asp Phe Gly Thr Tyr Pro Phe Val Thr Ser Ser Ser Pro Ser Ala
290 295 300

Gly Gly Ile Cys Thr Gly Leu Gly Ile Ala Pro Arg Ala Ile Gly Asp
305 310 315 320

Leu Ile Gly Val Val Lys Ala Tyr Thr Ser Arg Val Gly Ser Gly Pro
325 330 335

Phe Pro Thr Glu Leu Phe Gly Glu Glu Gly Asp Arg Leu Arg Lys Ala
340 345 350

Gly Met Glu Phe Gly Thr Thr Thr Gly Arg Pro Arg Arg Cys Gly Trp
355 360 365

Leu Asp Ile Val Ala Leu Lys His Ser Cys Gln Ile Asn Gly Phe Ser
370 375 380

Ser Leu Asn Leu Thr Lys Leu Asp Val Leu Ser Gly Leu Ser Glu Ile
385 390 395 400

Lys Val Gly Val Ser Tyr Thr Gln Thr Asp Gly Gln Lys Leu Gln Ser
405 410 415

Phe Pro Gly Asp Leu Asp Thr Leu Glu Gln Val Gln Val Asn Tyr Glu
420 425 430

Val Leu Pro Gly Trp Gln Ser Asp Ile Ser Ser Val Arg Arg Tyr Asp
435 440 445

Glu Leu Pro Gln Ala Ala Arg Leu Tyr Val Glu Arg Ile Glu Glu Leu
450 455 460

Val Gly Val Pro Val His Tyr Ile Gly Val Gly Pro Gly Arg Asp Ala
465 470 475 480

Leu Ile Tyr Lys

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1741 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1428
(D) OTHER INFORMATION: /product= "Wheat Adenylosuccinate Synthetase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GCC GCC GCC GCC GCC GGG CGG GGG AGG TCC TTC TCC CCG GCC GCC CCG        48
Ala Ala Ala Ala Ala Gly Arg Gly Arg Ser Phe Ser Pro Ala Ala Pro
 1               5                  10                  15

GCG CCG TCG TCG GTG CGC CTG CCC GGG AGA CAG GCC CCC GCC CCC GCC        96
Ala Pro Ser Ser Val Arg Leu Pro Gly Arg Gln Ala Pro Ala Pro Ala
            20                  25                  30

GCC GCG TCC GCG CTC GCG GTG GAG GCG GAC CCC GCC GCC GAC AGG GTC       144
Ala Ala Ser Ala Leu Ala Val Glu Ala Asp Pro Ala Ala Asp Arg Val
        35                  40                  45

TCG TCG CTG AGC CAG GTC TCC GGC GTG CTC GGG TCG CAG TGG GGC GAC       192
Ser Ser Leu Ser Gln Val Ser Gly Val Leu Gly Ser Gln Trp Gly Asp
    50                  55                  60

GAG GGG AAG GGG AAG CTC GTC GAC GTG CTC GCC CCC CGC TTC GAC ATC       240
Glu Gly Lys Gly Lys Leu Val Asp Val Leu Ala Pro Arg Phe Asp Ile
65                  70                  75                  80

GTC GCG CGT TGC CAG GGT GGA GCA AAT GCT GGA CAC ACC ATC TAC AAC       288
Val Ala Arg Cys Gln Gly Gly Ala Asn Ala Gly His Thr Ile Tyr Asn
```

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                    85                  90                  95

TCT GAA GGC AAG AAA TTT GCC CTT CAT CTT GTT CCA TCT GGT ATT CTC      336
Ser Glu Gly Lys Lys Phe Ala Leu His Leu Val Pro Ser Gly Ile Leu
            100                 105                 110

CAT GAA GGA ACA CTC TGT GTT GTT GGC AAC GGA GCG GTG ATC CAT GTT      384
His Glu Gly Thr Leu Cys Val Val Gly Asn Gly Ala Val Ile His Val
        115                 120                 125

CCA GGG TTC TTT GGC GAA ATT GAT GGT CTT CAA TCA AAT GGA GTC AGT      432
Pro Gly Phe Phe Gly Glu Ile Asp Gly Leu Gln Ser Asn Gly Val Ser
    130                 135                 140

TGT GAT GGA AGA ATA CTG GTG TCT GAC AGG GCT CAT TTG CTC TTT GAT      480
Cys Asp Gly Arg Ile Leu Val Ser Asp Arg Ala His Leu Leu Phe Asp
145                 150                 155                 160

CTG CAT CAG ACT GTA GAT GGA CTT AGG GAA GCC GAG CTT GCA AAT TCC      528
Leu His Gln Thr Val Asp Gly Leu Arg Glu Ala Glu Leu Ala Asn Ser
                165                 170                 175

TTC ATA GGA ACG ACT AAG AGA GGC ATT GGA CCT TGT TAT TCC AGC AAG      576
Phe Ile Gly Thr Thr Lys Arg Gly Ile Gly Pro Cys Tyr Ser Ser Lys
            180                 185                 190

GTC ACT CGA AAT GGG CTG CGA GTT TGT GAT CTA AGG CAC ATG GAC ACT      624
Val Thr Arg Asn Gly Leu Arg Val Cys Asp Leu Arg His Met Asp Thr
        195                 200                 205

TTT GGG GAT AAG CTT GAT GTT TTA TTC GAA GAT GCT GCT GCG AGG TTT      672
Phe Gly Asp Lys Leu Asp Val Leu Phe Glu Asp Ala Ala Ala Arg Phe
    210                 215                 220

GAA GGC TTC AAG TAC AGC AAA GGC ATG CTC AAG GAA GAG GTT GAG AGG      720
Glu Gly Phe Lys Tyr Ser Lys Gly Met Leu Lys Glu Glu Val Glu Arg
225                 230                 235                 240
```

```
TAC AAG AGG TTT GCA GAG CGT TTG GAG CCC TTC ATT GCT GAC ACT GTT      768
Tyr Lys Arg Phe Ala Glu Arg Leu Glu Pro Phe Ile Ala Asp Thr Val
                245                 250                 255

CAT GTG TTG AAT GAA TCC ATC CGA CAG AAG AAG AAA ATT CTG GTT GAA      816
His Val Leu Asn Glu Ser Ile Arg Gln Lys Lys Lys Ile Leu Val Glu
            260                 265                 270

GGT GGT CAG GCA ACT ATG CTG GAT ATC GAT TTT GGA ACT TAT CCA TTT      864
Gly Gly Gln Ala Thr Met Leu Asp Ile Asp Phe Gly Thr Tyr Pro Phe
        275                 280                 285

GTG ACT TCT TCT AGC CCT TCC GCT GGT GGA ATT TGC ACT GGC CTT GGG      912
Val Thr Ser Ser Ser Pro Ser Ala Gly Gly Ile Cys Thr Gly Leu Gly
    290                 295                 300

ATT GCC CCT AGG GTT ATT GGC GAC CTG ATT GGA GTT GTA AAA GCT TAC      960
Ile Ala Pro Arg Val Ile Gly Asp Leu Ile Gly Val Val Lys Ala Tyr
305                 310                 315                 320

ACA ACA AGG GTT GGC TCT GGC CCT TTC CCA ACT GAA CTG CTT GGA GAG      1008
Thr Thr Arg Val Gly Ser Gly Pro Phe Pro Thr Glu Leu Leu Gly Glu
                325                 330                 335

GAA GGT GAT GTT CTT AGG AAG GCC GGA ATG GAA TTT GGA ACG ACT ACA      1056
Glu Gly Asp Val Leu Arg Lys Ala Gly Met Glu Phe Gly Thr Thr Thr
            340                 345                 350

GGT CGC CCA AGA CGT TGT GGC TGG CTT GAC ATC GTT GCA CTG AAA TAC      1104
Gly Arg Pro Arg Arg Cys Gly Trp Leu Asp Ile Val Ala Leu Lys Tyr
        355                 360                 365

TGC TGT GAC ATC AAT GGG TTT TCC TCT CTA AAT CTA ACA AAA CTT GAT      1152
Cys Cys Asp Ile Asn Gly Phe Ser Ser Leu Asn Leu Thr Lys Leu Asp
    370                 375                 380
```

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GTT CTG TCC GGG TTA CCA GAA ATT AAG CTG GGT GTT TCT TAT AAT CAA      1200
Val Leu Ser Gly Leu Pro Glu Ile Lys Leu Gly Val Ser Tyr Asn Gln
385                 390                 395                 400

ATG GAT GGA GAG AAA CTA CAA TCC TTC CCA GGG GAT CTT GAC ACC CTG      1248
Met Asp Gly Glu Lys Leu Gln Ser Phe Pro Gly Asp Leu Asp Thr Leu
                405                 410                 415

GAG CAA GTA CAG GTC AAC TAT GAG GTG CTT CCT GGG TGG GAC AGT GAC      1296
Glu Gln Val Gln Val Asn Tyr Glu Val Leu Pro Gly Trp Asp Ser Asp
            420                 425                 430

ATA TCT TCT GTC CGA AGT TAC AGT GAA CTC CCC CAA GCT GCC CGC CGT      1344
Ile Ser Ser Val Arg Ser Tyr Ser Glu Leu Pro Gln Ala Ala Arg Arg
        435                 440                 445

TAC GTG GAG AGG ATA GAA GAG CTC GCC GGT GTT CCA GTC CAC TAC ATT      1392
Tyr Val Glu Arg Ile Glu Glu Leu Ala Gly Val Pro Val His Tyr Ile
    450                 455                 460

GGT GTC GGG CCT GGG AGG GAT GCT CTG ATA TAC AAG TAAAGGGCAA          1438
Gly Val Gly Pro Gly Arg Asp Ala Leu Ile Tyr Lys
465                 470                 475

ACTCGATTTG GTACTATTGT ATCGGACGAA ATAATTCAGT CTTAACTAGG CCGTTGTGAG    1498

CATTGCTGTG TCAGCACACC CTTGATTGCC AATCGTAGCG GGTAATACGA TCGACAAGCT    1558

ACTGGCGGGC GGGGTGATGT AATACCTGCA ATAATGATTT CCGGGAAATG TCCCGATATA    1618

TCACCATAAG GATGCAGTGT TAGAGTTTGG TGGTAACATT TTGTCTTTCG ACTCCACCAA    1678

TGGTTTGGTG GTATTATCAC AATTCACCGT CAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    1738

AAA                                                                 1741
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ala Ala Ala Ala Ala Gly Arg Gly Arg Ser Phe Ser Pro Ala Ala Pro
 1               5                  10                  15

Ala Pro Ser Ser Val Arg Leu Pro Gly Arg Gln Ala Pro Ala Pro Ala
            20                  25                  30

Ala Ala Ser Ala Leu Ala Val Glu Ala Asp Pro Ala Ala Asp Arg Val
        35                  40                  45

Ser Ser Leu Ser Gln Val Ser Gly Val Leu Gly Ser Gln Trp Gly Asp
    50                  55                  60

Glu Gly Lys Gly Lys Leu Val Asp Val Leu Ala Pro Arg Phe Asp Ile
65                  70                  75                  80

Val Ala Arg Cys Gln Gly Gly Ala Asn Ala Gly His Thr Ile Tyr Asn
                85                  90                  95

Ser Glu Gly Lys Lys Phe Ala Leu His Leu Val Pro Ser Gly Ile Leu
            100                 105                 110

His Glu Gly Thr Leu Cys Val Val Gly Asn Gly Ala Val Ile His Val
        115                 120                 125

Pro Gly Phe Phe Gly Glu Ile Asp Gly Leu Gln Ser Asn Gly Val Ser
    130                 135                 140
```

BNSDOCID: <WO 9619576A1 I >

Cys Asp Gly Arg Ile Leu Val Ser Asp Arg Ala His Leu Leu Phe Asp
145 150 155 160

Leu His Gln Thr Val Asp Gly Leu Arg Glu Ala Glu Leu Ala Asn Ser
165 170 175

Phe Ile Gly Thr Thr Lys Arg Gly Ile Gly Pro Cys Tyr Ser Ser Lys
180 185 190

Val Thr Arg Asn Gly Leu Arg Val Cys Asp Leu Arg His Met Asp Thr
195 200 205

Phe Gly Asp Lys Leu Asp Val Leu Phe Glu Asp Ala Ala Ala Arg Phe
210 215 220

Glu Gly Phe Lys Tyr Ser Lys Gly Met Leu Lys Glu Glu Val Glu Arg
225 230 235 240

Tyr Lys Arg Phe Ala Glu Arg Leu Glu Pro Phe Ile Ala Asp Thr Val
245 250 255

His Val Leu Asn Glu Ser Ile Arg Gln Lys Lys Lys Ile Leu Val Glu
260 265 270

Gly Gly Gln Ala Thr Met Leu Asp Ile Asp Phe Gly Thr Tyr Pro Phe
275 280 285

Val Thr Ser Ser Ser Pro Ser Ala Gly Gly Ile Cys Thr Gly Leu Gly
290 295 300

Ile Ala Pro Arg Val Ile Gly Asp Leu Ile Gly Val Val Lys Ala Tyr
305 310 315 320

Thr Thr Arg Val Gly Ser Gly Pro Phe Pro Thr Glu Leu Leu Gly Glu
325 330 335

BNSDOCID: <WO 9619576A1 I >

Glu Gly Asp Val Leu Arg Lys Ala Gly Met Glu Phe Gly Thr Thr Thr
340 345 350

Gly Arg Pro Arg Arg Cys Gly Trp Leu Asp Ile Val Ala Leu Lys Tyr
355 360 365

Cys Cys Asp Ile Asn Gly Phe Ser Ser Leu Asn Leu Thr Lys Leu Asp
370 375 380

Val Leu Ser Gly Leu Pro Glu Ile Lys Leu Gly Val Ser Tyr Asn Gln
385 390 395 400

Met Asp Gly Glu Lys Leu Gln Ser Phe Pro Gly Asp Leu Asp Thr Leu
405 410 415

Glu Gln Val Gln Val Asn Tyr Glu Val Leu Pro Gly Trp Asp Ser Asp
420 425 430

Ile Ser Ser Val Arg Ser Tyr Ser Glu Leu Pro Gln Ala Ala Arg Arg
435 440 445

Tyr Val Glu Arg Ile Glu Glu Leu Ala Gly Val Pro Val His Tyr Ile
450 455 460

Gly Val Gly Pro Gly Arg Asp Ala Leu Ile Tyr Lys
465 470 475

BNSDOCID: <WO 9619576A1 I >

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 8-17

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution: Agricultural Research Service Culture Collection (NRRL)

Address of depositary institution *(including postal code and country)*:
1815 North University Street
Peoria, IL 61604
USA

Date of deposit	Accession Number
22 September 1994 (22.09.94)	B-21328

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

We request the Expert Solution where available

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international applicati n	☐ This sheet was received by the International Bureau on:
Authorized fficer C.A.J.A. PASCHE	Authorized officer

Form PCT/RO/134 (July 1992)

BNSDOCID: <WO 9619576A1 I >

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 8-17

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution: Agricultural Research Service Culture Collection (NRRL)

Address of depositary institution *(including postal code and country)*:
1815 North University Street
Peoria, IL 61604
USA

Date of deposit	Accession Number
24 October 1994 (24.10.94)	B-21349

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

We request the Expert Solution where available

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international applicati n	☐ This sheet was received by the International Bureau on:
Authorized officer: C.A.J.A. PASCHE	Authorized officer

Form PCT/RO/134 (July 1992)

BNSDOCID: <WO 9619576A1 I >

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 8-17

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution: Agricultural Research Service Culture Collection (NRRL)

Address of depositary institution *(including postal code and country)*:
1815 North University Street
Peoria, IL 61604
USA

Date of deposit	Accession Number
03 November 1995 (03.11.95)	B-21505

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

We request the Expert Solution where available

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international applicati n	☐ This sheet was received by the International Bureau on:
Authorized officer: C.A.M.A. PASCHE	Authorized officer

BNSDOCID: <WO 9619576A1 >

We claim:

1. An isolated DNA molecule encoding a protein from a plant having adenylosuccinate synthetase(ADSS) activity.

2. The isolated DNA molecule of claim 1, wherein said plant is a dicotyledon.

3. The isolated DNA molecule of claim 2, wherein said dicotyledon is an *Arabidopsis* species.

4. The isolated DNA molecule of claim 3, wherein said protein comprises the amino acid sequence set forth in SEQ ID NO: 2.

5. The isolated DNA molecule of claim 4 comprising the sequence set forth in SEQ ID NO: 1.

6. The isolated DNA molecule of claim 1, wherein said plant is a monocotyledon.

7. The isolated DNA molecule of claim 6, wherein said monocotyledon is selected from the group consisting of maize and wheat.

8. The isolated DNA molecule of claim 7, wherein said monocotyledon is maize.

9. The isolated DNA molecule of claim 8, wherein said protein comprises the amino acid sequence set forth in SEQ ID NO: 4.

10. The isolated DNA molecule of claim 9 comprising the sequence set forth in SEQ ID NO: 3.

11. The isolated DNA molecule of claim 7 wherein said monocotyledon is wheat.

12. The isolated DNA molecule of claim 11, wherein said protein comprises th amino acid sequence set forth in SEQ ID NO: 6.

13. The isolated DNA molecule of claim 12 comprising the sequence set forth in SEQ ID NO: 5.

14. An expression cassette comprising a promoter operably linked to the DNA molecule of any one of claims 1 to 13.

15. A recombinant vector comprising the expression cassette of claim 14, wherein said vector is capable of being stably transformed into a host cell.

16. A host cell stably transformed with the vector of claim 15, wherein said host cell is capable of expressing said DNA molecule.

17. A host cell of claim 16 selected from the group consisting of a bacterial cell, a yeast cell, and an insect cell.

18. A method for assaying a chemical for the ability to inhibit the activity of an ADSS enzyme from a plant comprising

(a) combining said ADSS enzyme in a first reaction mixture under conditions in which said ADSS enzyme is capable of catalyzing the synthesis of adenylosuccinate;

(b) combining said chemical and said ADSS enzyme in a second reaction mixture under the same conditions as in said first reaction mixture; and

(d) comparing the amount of adenylosuccinate produced in said first and said second reaction mixture;

wherein said chemical is capable of inhibiting the activity of said ADSS enzyme if the amount of adenylosuccinate in said second reaction mixture is significantly less than the amount of adenylosuccinate in said first reaction mixture.

19. A nucleotide probe capable of specifically hybridizing to a plant adenylosuccinate synthetase gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an adenylosuccinate synthetase enzyme from a plant at least 10 nucleotides in length.

20. A method of producing a protein having adenylosuccinate synthetase(ADSS) activity in a host organism comprising

(a) inserting a DNA sequence encoding a protein having adenylosuccinate synthetase(ADSS) activity into an expression cassette designed for the chosen host;

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(b) inserting the resultant molecule, containing the individual elelments linked in proper reading frame, into a vector capable of being transformed into the host cell;
(c) growing the thus transformed host cell in a suitable culture medium; and
(d) isolating the protein product either from the transformed cell or the culture medium or both and purifying it.

21. A method of producing a DNA molecule comprising a DNA portion encoding a protein having adenylosuccinate synthetase(ADSS) activity comprising

(a) preparing a nucleotide probe capable of specifically hybridizing to a plant adenylosuccinate synthetase gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an adenylosuccinate synthetase enzyme from a plant at least 10 nucleotides in length;

(b) probing for other ADSS coding sequences in popluations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a) ; and

(c) isolating a DNA molecule comprising a DNA portion encoding a protein having adenylosuccinate synthetase(ADSS) activity.

22. Use of a nucleotide probe according to claim 19 to amplify and/or analyse ADSS coding sequences from a chosen organism via the process of polymerase chain reaction (PCR).

23. Use of a nucleotide probe according to claim 19 to map the location of the native ADSS gene(s) in the genome of a chosen plant using standard techniques based on the selective hybridization of the probe to genomic ADSS sequences.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04880

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/52 C12N1/21 C12N15/[redacted] C12Q1/25
C12P21/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHYTOCHEMISTRY, vol. 6, 1967 pages 115-119, M.D. HATCH; 'Inhibition of plant adenylosuccinate synthetase by hadacidin and the mode of action of hadacidin and structurally related compounds on plant growth' see the whole document. --- -/--	1,6,11, 18,20

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1996	17.05.96

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C.(Continuation) DOCUMENTS CONSID[redacted]TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DDBJ Database entry OS0501A, accession number D15352, 17 May 1993. see sequence. & PLANT J., vol. 6, 1994 pages 615-624, T. SASAKI ET AL.; 'Towarscataloguing all rice genes: large-scale sequencing of randomly chosen rice cDNAs from a callus cDNA library' ---	19
X	FEBS LETT., vol. 303, 1992 pages 4-10, S.M. POWELL ET AL.; 'Cloning and characterization of the cDNA encoding human adenylosuccinate synthetase' cited in the application see Introduction and Experimental sections and page 9. ---	20
A	GENETICS, vol. 120, 1988 pages 1111-1124, D.P. SNUSTAD ET AL.; 'Maize glutamine synthetase cDNAs: isolation by direct genetic selection in Escherichia coli' cited in the application see abstract, introduction and the first paragraph of the discussion. -----	1